Immuno-gold labelling

Protein A-gold and antibody-gold conjugates are usually stable for at least 3 months; if labelling efficiency has decreased, the gold-conjugates can be washed with PBS, 0.1% BSA to remove competing desorbed IgG/Protein A.

Samples on carbon-coated formvar copper grids are floated face down on sequential drops of labelling and washing solutions. Typically, 100μl drops are used per 2 grids for all solutions except antibody solutions where only 5μl per grid is sufficient. The labelling is performed on fresh Parafilm stuck to the bench using the capillary action of water; care must be taken not to touch the surface of the Parafilm with ungloved hands or anything that will contaminate the solutions.

Never let the grid become wet on the back surface or dry out during the incubation procedure.

1. Quench 0.02 M glycine in PBS- to quench free aldehyde groups (3x2 min)
2. Block (0.2% fish skin gelatin, 0.2% BSA (Sigma Type V) in PBS) (10 min)
3. Specific antibody (5-20μg/ml) diluted in Block and pre-spun in microfuge for 5 minutes, max speed. (≥ 30 min)

Note: Double labelling can performed by co-incubating two different species antibodies if individual optimal antibody concentrations have been empirically determined previously.

4. Wash on Block (5x3 min)

Note: If specific antibodies with weak binding capacity for Protein A are used, like sheep and goat IgG and some monoclonals, or when an enhancement of the gold signal is desired an additional step can be included using a bridging antibody (rabbit anti goat, sheep or mouse; swine anti rabbit) diluted in Block followed by washing (step 4).

5. Incubate with protein A-gold or secondary antibody-gold, diluted in Block (often 1:50-1:100). Gold probe dilutions are made fresh and used immediately. (≥ 30 min)
6. Wash on Block (5x3 min)

See optional steps 6a-e below for double labelling with primaries from same species.

7. Wash with de-ionized water (fresh, not from plastic bench-bottles) to remove phosphate ions. Wash forceps in de-ionized water between each step. (5x2 min)
8. Incubate on ice cold 1.8% methylcellulose (25Ctp)/0.3% uranyl acetate (MC-UA) (10 min)
9. Pick up the grids with 4-5mm wire loops, reduce the MC-UA to an even thin film by drawing the edge of the loop along a piece of filter paper, dry in dust-free environment.

Resin embedded sections are incubated in the same way, except that:

I. BSA may be replaced by 5-10% fetal calf serum (FCS), or a 10 min incubation on PBS/FCS prior to the procedure may be added.
II. Antibody and protein A/gold incubation periods are prolonged to ≥ 60 min.
III. Washing in distilled water (step 7) is prolonged to ≥ 60 min.
IV. Osmium and/or lead contrasting methods usually replace the MC-UA embedding steps 8 and 9.

Vesicles and cell fractions

When immunolabelling vesicles or whole mounts of intracellular membrane preparations, a drop of the preparation is placed on the Parafilm. Dry grids are then placed, film side down, on the drops and left for 10min for the vesicles to adhere. The grids are then removed, fixed (e.g. 4% paraformaldehyde, 0.1% glutaraldehyde in PBS) and treated as above.

OPTIONAL STEPS 6a-e.

If the sample is to be double labelled with two primary antibodies derived from the same species, a glutaraldehyde fixation step will prevent the second round of primary and secondary labelling from non-specifically binding the first round of label. The experiment needs to be carefully controlled since the glutaraldehyde step doesn't always produce complete blocking of the first round of antibody labelling.

6a. Wash on PBS (2x1 min)
6b. Stabilise the reaction on 1% glutaraldehyde in PBS (5 min)
6c. Wash on PBS (2x5 min)
6d. In case of double labelling, quench fix with (0.02M glycine, PBS) (5x2min)
6e. Repeat steps 3-6 with different 1°/2° antibody combination after a 5x2 min rinse with PBS/glycine.

Remedies for high non-specific labelling problems:

I. Make antibody concentrations more dilute, pre-spin to remove antibody aggregates.
II. Glutaraldehyde generally gives a higher background than paraformaldehyde. If possible exclude glutaraldehyde from the fixation buffer. Note: paraformaldehyde fixation is partially reversible so long wash steps should be avoided.
III. Acetylated BSA has a much higher binding affinity that BSA and will block non-specific binding more stringently.